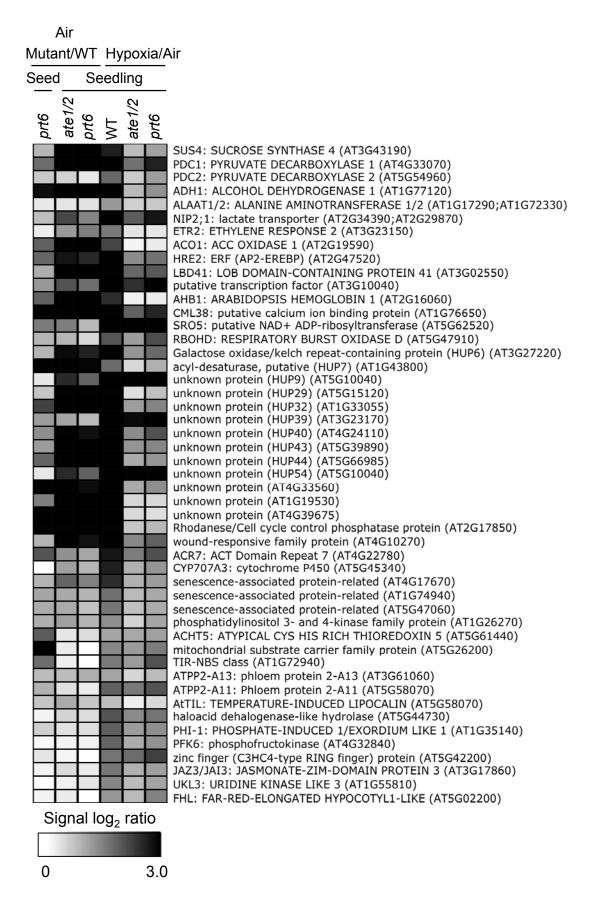


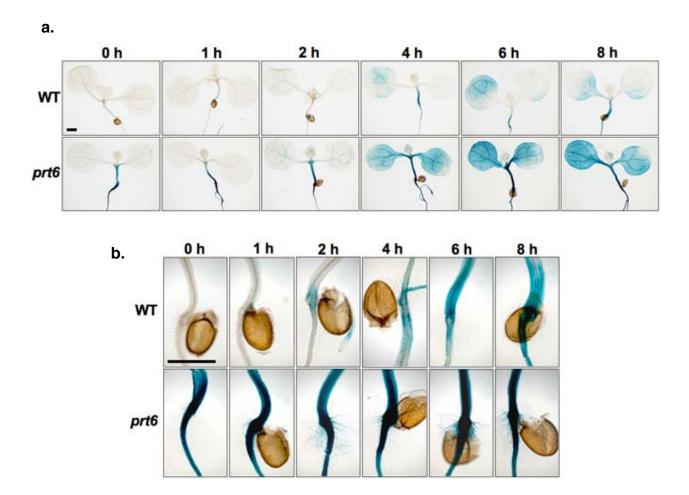
Supplementary Fig. 1. Diagrammatic representation of the N-end rule pathway of targeted proteolysis (after Graciet and Wellmer 2010⁹).

Tertiary, secondary and primary destabilising residues are highlighted, referring to the number of steps required before recognition for proteosomal degradation. Primary destabilising residues associated with PRT6 are boxed in red.

Coloured ovals represent protein substrates. Single letter amino acid codes are shown for N-terminal residues. MAP, Methionine Amino Peptidase; C* represents oxidised C; NTAN1 and NTAQ1, Nt-amidases for Asn and Gln; ATE, Arginyl tRNA Transferase; PRT, Proteolysis (E3 ligase).



Supplementary Fig. 2. Expression data for the 49 core anaerobic response genes recognized by evaluation of seedling root and shoot cell-type specific mRNAs¹⁵. The analysis compares transcript accumulation in WT (Col-0) and mutants under air or hypoxia $(2h - O_2)$. Column order is the same as in Figure 1a. Values are Signal Log₂Ratio (SLR) data from Supplementary Table 1.



Supplementary Fig. 3. Time course analysis of *pADH1::GUS* expression in Col-0 (WT) and *prt6* mutant 7 day old seedlings. Time (h) after transfer to hypoxic conditions is indicated for each sample. The *pADH1::GUS* transgene was crossed into the *prt6* background and seedlings homozygous for *prt6* and the transgene were assayed.

- a. Imaging of whole seedlings
- b. Imaging of the root/shoot junction and seed coat. Bar indicates $100\mu m$

a.

	10	ERF Grou
RAP2.12	MCGGAIISDFIPPP	VII
HRE1	MCGGAVISDYIAPE	VII
HRE2	MCGGAIISDFIWSK	VII
RAP2.2	MCGGAIISDFIPPP	VII
RAP2.3	MCGGAIISDYAP	VII
Os11g06770.2	MCGGGKVASPPGPR	X
Os06g43220.1	MCRDCGKOVYLGGF	
Os08g43210.1	MCTSKLEEITGEWP	III
Os09g35030.1	MCGIK-OEMSGESS	III
Os03g08490.1	MCGGAILAEFIPAP	VII
Os03g08500.1	MCGGAILAELIPSA	VII
Os03g08470.1	MCGGATLANIIPAT	VII
Os09g26420.1	MCGGAIISGFIPPS	VII
Os06g09390.1	MCGGATLSDLIPPP	VII
Os02g54160.1	MCGGAITHHLKGHP	VII
Os03g22170.1	MCGGAIPLISSR	VII
Os07g47790.1	MCGGAIISDFIPOR	VII
Os01g21120.1	MCGGAIIYDYIPAR	VII
Os05g29810.1	MCGGAIIADFVPPA	VII
Os12g41060.1	MCGGGPDNHHAITV	XI
SNORKEL1	MCGGCLIPDELVGK	VII
SNORKEL2	MCGENDNNGAAAGS	VII
SUB1A-1	MCGGEVIPADMPAA	VII
SUB1B-1	MCGGALIPNDYGDK	VII
SUB1C-1	MRRRV	VII
	MCGGAII	,
	TIOOOTILL T	

b. 10
MAF5 MCRKSEAMGRRRVE
VRN2 MCRQNCRAKSSPEE

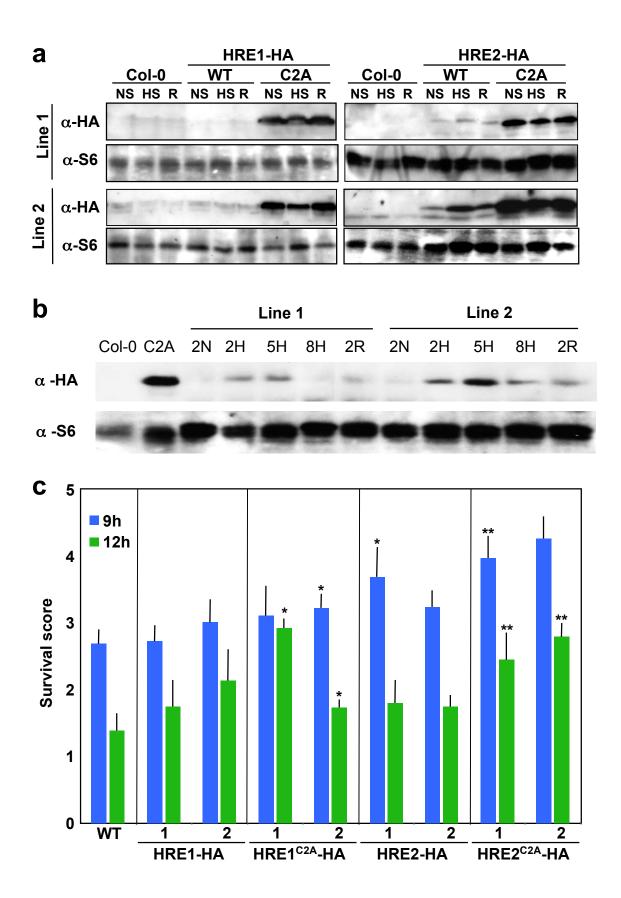
Supplementary Fig. 4. Arabidopsis and rice ethylene responsive factor proteins with a MC-N terminus.

- a. ClustalW³⁷ alignment of Arabidopsis (At) and rice (Os) ERFs with a N-terminal MC-dipeptide. ERF group assignments are from Nakano *et al*¹⁷.
- ERF group assignments in red are based on phylogenetic characterization of the SK and SUB1 genes²¹. Conserved residues are shaded.

An aspartic acid (E, red box) of SUB1A-1 was the target of mutation.

b. N-terminal sequences of MADS AFFECTING FLOWERING 5 (MAF5) and REDUCED VERNALIZATION RESPONSE 2 (VRN2) that were used as controls in this study.

Supplemental Figure 5: HRE proteins are stabilized under low oxygen and Improve survival of hypoxia (*legend on next page*)



Supplemental Figure 5: HRE proteins are stabilized under low oxygen and Improve survival of hypoxia

- a. *In vivo* stability of WT and C2A variants of HRE1-HA and HRE2-HA under non-stress (NS), 2 h hypoxic stress (HS) and after 1 h recovery from 2 h HS (R) in 7-d-old Arabidopsis seedlings. Data for each transgene are shown for two independent lines (1 and 2). Equal amounts of protein were loaded in each gel lane and ribosomal protein S6 (S6) was used as a loading control.
- b. A time course of *in vivo* stability of WT and C2A variants of HRE2-HA under non-stress 2 h (N), 2, 5 and 8 h hypoxic stress (H) and after 2 h recovery from 8 h HS (2R) in 7-d-old Arabidopsis seedlings. Data shown for two independent lines (1 and 2). Equal amounts of protein were loaded in each gel lane and ribosomal protein S6 (S6) was used as a loading control.
- c. Survival data of 7-day old seedlings expressing WT or C2A variants of HRE1-HA and HRE2-HA after 9 h or 12 h hypoxic stress. Data for each transgene are shown for two independent lines (1 and 2). Data represent mean \pm SD from a representative experiment of three biological replicates. An asterisk indicates a significant difference between the transgenic and WT seedlings grown on the same plate (* = P <0.05, ** = P < 0.01).